

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
United States Patent and Trademark  
Office  
Box PCT  
Washington, D.C.20231  
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 October 2000 (03.10.00)	
International application No. PCT/NL00/00117	Applicant's or agent's file reference BO 42503
International filing date (day/month/year) 24 February 2000 (24.02.00)	Priority date (day/month/year) 24 February 1999 (24.02.99)
Applicant JETTEN, Jan, Matthijs et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:28 August 2000 (28.08.00)☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Pascal Piriou Telephone No.: (41-22) 338.83.38
---	---

02/914/182

Copy for the Elected Office (EO/US)

PCT/NL00/00117

## PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

JORRITSMA, Ruurd  
Nederlandsch Octrooibureau  
Scheveningseweg 82  
P.O. Box 29720  
NL-2502 LS The Hague  
PAYS-BAS

Date of mailing (day/month/year) 28 August 2001 (28.08.01)	<b>IMPORTANT NOTIFICATION</b>
Applicant's or agent's file reference EP9805957	
International application No. PCT/NL00/00117	International filing date (day/month/year) 24 February 2000 (24.02.00)

1. The following indications appeared on record concerning:

☒ the applicant ☒ the inventor ☐ the agent ☐ the common representative

Name and Address VAN WANDELEN, Mario, Tarcisius, Ragmandus Roosenveltlaan 14 NL-3705 PG Zeist Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address VAN WANDELEN, Mario, Tarcisius, Ragmandus Rooseveltlaan 14 NL-3705 PG Zeist Netherlands	State of Nationality NL	State of Residence NL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Anman QIU Telephone No.: (41-22) 338.83.38
---	---

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT


(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>BO 42503</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/NL00/00117</b>	International filing date (day/month/year) <b>24/02/2000</b>	Priority date (day/month/year) <b>24/02/1999</b>
International Patent Classification (IPC) or national classification and IPC <b>C12P1/00</b>		
Applicant <b>NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-... et al</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.  
  
☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
  
 These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand <b>28/08/2000</b>	Date of completion of this report <b>06.06.2001</b>
Name and mailing address of the international preliminary examining authority:   <b>European Patent Office</b> <b>D-80298 Munich</b> <b>Tel. +49 89 2399 - 0 Tx: 523656 epmu d</b> <b>Fax: +49 89 2399 - 4465</b>	Authorized officer  <b>Douschan, K</b>  Telephone No. +49 89 2399 8702



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL00/00117

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, pages:**

1-9 as originally filed

**Claims, No.:**

1-12 as received on 19/04/2001 with letter of 19/04/2001

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL00/00117

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**II. Priority**

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
  - ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:  
**see separate sheet**

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:
- ☒ restricted the claims.
  - ☐ paid additional fees.
  - ☐ paid additional fees under protest.
  - ☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☒ complied with.
  - ☐ not complied with for the following reasons:
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL00/00117

- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-12.

## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

### 1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	12
Inventive step (IS)	Yes:	Claims	1-9, 10part., 11
	No:	Claims	10part., 12
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

### 2. Citations and explanations see separate sheet

## VI. Certain documents cited

### 1. Certain published documents (Rule 70.10)

and / or

### 2. Non-written disclosures (Rule 70.9)

see separate sheet

## VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:  
see separate sheet

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:  
see separate sheet

**Re Item IV**

**Lack of unity of invention**

It is stated that, upon invitation by the IPEA, the applicant restricted the claimed subject-matter to the embodiments of invention 1 (=claims 1-12), and postpones the adaption of the description until the entry into the national phase. Therefore the substantive examination of the present patent application covers only the subject-matter relating to claims 1-12. The corresponding passages of the description relating to claims 13-18 have not been taken into consideration.

Since the applicant has restricted the subject-matter claimed to one invention only, the requirements for unity of invention (cf. Rule 13 PCT) are met.

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

- 1). The documents mentioned in the international search report are cited by the following abbreviations:

- D1: CHEMICAL ABSTRACTS, vol. 100, no. 25, 18 June 1984 (1984-06-18)  
Columbus, Ohio, US; abstract no. 208639, SEMMELHACK, M. F. ET AL:  
'Oxidation of alcohols to aldehydes with oxygen and cupric ion, mediated by  
nitrosonium ion' & J. AM. CHEM. SOC. (1984), 106(11), 3374-6 ,
- D2: BOBBITT J M ET AL: 'ORGANIC NITROSONIUM SALTS AS OXIDANTS IN  
ORGANIC CHEMISTRY' HETEROCYCLES, vol. 27, no. 2, 1 January 1988  
(1988-01-01), pages 509-533,
- D3: NOOY DE A E J ET AL: 'HIGHLY SELECTIVE TEMPO MEDIATED  
OXIDATION OF PRIMARY ALCOHOL GROUPS IN POLYSACCHARIDES'  
RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS,NL,ELSEVIER  
SCIENCE PUBLISHERS. AMSTERDAM, vol. 113, no. 3, 1 March 1994  
(1994-03-01), pages 165-166,
- D4: NOOY DE A E J ET AL: 'ON THE USE OF STABLE ORGANIC NITROXYL  
RADICALS FOR THE OXIDATION OF PRIMARY AND SECONDARY  
ALCOHOLS' SYNTHESIS,DE,GEORG THIEME VERLAG. STUTTGART, 1  
October 1996 (1996-10-01), pages 1153-1174, and

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL00/00117

D5: WO 99 23117 A (VALTON TEKNILLINEN TUTKIMUSKESKUS) 14 May 1999 (1999-05-14).

D1 is the abstract of a scientific article (J. Am. Chem. Soc., 1984, 106 (11), 3374-6), which is used in the present IPER under the same numbering (=D1).

D5 is an intermediate document (cf. item VI below), which was published between priority date and filing date of the present patent application. Therefore D5 represents relevant state of the art for each subject of the present application, that goes beyond the content of the priority document (EP 99200536.3, 24.02.99), during the international phase. Since the said priority document is silent about the subject-matter of claim 12 and the feature "glyconic acid" in claim 10, the priority document is not regarded as being valid for claim 10 part. and claim 12, and consequently the content of D5 is taken into consideration for the assessment of novelty and inventive step of the subject-matter of the said two claims.

Moreover, a warning is given to the applicant that, in the regional phase before the European Patent Office, D5 may also play a role for the assessment of novelty for the whole claimed subject-matter, since it has been filed before the present priority date (cf. Art. 54(3) and 158(2) EPC).

- 2). The content of the present patent application and the prior art documents is discussed as follows:

The **present application** claims in **claims 1-11** a process for the oxidation of an alcohol (part of a carbohydrate or steroid) using a nitroxyl compound, like TEMPO, DOXYL, PROXYL, etc., and an oxidising agent in the presence of either an enzyme or a metal complex or both, in an aqueous medium, or in a mixture of water with alcohol, an ether or a water-immiscible organic solvent. The term "metal complex" in the claims is not specified in accordance with p. 3 of the description.

**Claim 12** claims the use of the process according to the other claims in the oxidation of OH-groups contained in cotton fibres, to introduce aldehyde groups.

**D1, D2 and D4** disclose a process for the preparation of nitrosonium ions by



oxidizing nitroxyl in the presence of copper ions (D1) or other metals - cf. D2 and D4, the resulting nitrosonium is used for the oxidation of alcohols to aldehydes. D4 also mentions aqueous and biphasic systems, but not in connection with enzymes or metal-complexes.

D3 concerns a process where TEMPO is oxidised to give a nitrosonium ion, which is used to oxidise primary alcohol groups in polysaccharides; metals or enzymes are not mentioned.

D5 discloses the same process as claimed in the present application, i.e. the oxidation of a nitroxyl compound (TEMPO) in the presence of an enzyme, and using the said nitrosonium ion for the oxidation of OH-groups in (cellulosic) textile fibres. As already mentioned above, D5 can be taken into consideration only for claim 10 part. and claim 12.

3). Novelty (Art. 33(1) and (2) PCT):

In the light of what is said under items 2.) above and taking into consideration that the objections raised under VIIIa) and VIIIb) below - with a view that the term "metal complex" is undefined - have to be met, the claimed subject-matter of claims 1-11 appears to be new.

With regard to claim 12, this claim is not entitled to the priority date and therefore D5 is relevant prior art. Since D5 concerns an identical process with regard to the use of an enzyme, performed in an aqueous medium, claim 12 lacks novelty.

4). Inventive step (Art. 33(1) and (3) PCT):

Claims 1-9, 10part. and 11:

None of D1-D4 suggests the use of an enzyme in the claimed process, nor is the use of the metal complexes as specified on p. 3 of the present description in combination with nitroxyl compounds obvious therefrom. Therefore the said claims appear to involve an inventive step.

Claims 10part. and 12:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL00/00117

Claim 12 is not new in the light of D5 (cf. item V-3), and therefore is also not inventive.

The feature "glyconic acid" in claim 10 is merely a preferred embodiment which is not entitled to the priority date of the present application. Since it is regarded as being obvious from D5 that also "glyconic acids" can be used as a carbohydrate substrate, no inventive step can be recognised for the said feature.

**Re Item VI**

**Certain documents cited**

**Certain published documents (Rule 70.10)**

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO 99 23117	14.05.99	04.11.98	04.11.97

**Re Item VII**

**Certain defects in the international application**

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

**Re Item VIII**

**Certain observations on the international application**

- a) The term "and/or" in claim 1 is confusing, contrary to Art. 6 PCT, and is interpreted as comprising the presence of an enzyme alone, the presence of a metal complex alone, or the presence of a metal complex and an enzyme.
- b) The term "metal complex" in the claims (cf. claim 1 ) is undefined. Since it is clear

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/NL00/00117

from the description on p. 3 that very specific, unusual metal complexes are meant, the said term should be specified in claim 1 (Art. 6 PCT). Moreover, it appears that the present process does not work with *any* common metal complex (Art. 5 PCT).

- c) The term "especially" used in the claims (cf. e.g. claims 2, 4) is not limiting, and therefore renders the scope of the said claims obscure (Art. 6 PCT). Therefore this term should be avoided in the claims, the feature mentioned after "especially" should be made the subject of a further dependent claim.

# ATENT COOPERATION TREAT

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>B0 42503</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/NL 00/ 00117</b>	International filing date (day/month/year) <b>24/02/2000</b>	(Earliest) Priority Date (day/month/year) <b>24/02/1999</b>
Applicant  <b>NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-... et al</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

### 1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

**PROCESS FOR PRODUCING NITROSONIUM IONS FROM A NITROXYL COMPOUND, THEIR APPLICATION IN THE SELECTIVE OXIDATION OF PRIMARY ALCOHOLS, AND NOVEL CARBOHYDRATE ALDEHYDES**

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NL 00/00117

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-12

Claims 1-6 concern a process for producing nitrosonium ions by oxidising a nitroxyl compound in the presence of an enzyme and/or a metal complex.

Claims 7-11 claim the use of the nitrosonium ions prepared in a process according to claims 1-6 for oxidising a primary alcohol, which is not specific and can be either from a carbohydrate or a steroid, etc.

Claim 12 describes the application of the process of claims 7-11 in a process for treating textile fibres.

2. Claims: 13-18

Claims 13-18 concern an oxidised carbohydrate having specific characteristics as defined in claim 13 and containing at least one "cyclic monosaccharide chain group carrying a carbaldehyde group". The said oxidised carbohydrate "can" be prepared by the process of claims 7-11 (cf. p. 4 of the description), but is not limited thereto. The oxidised carbohydrates may also be further processed or derivatised (see claims 16-18). The products claimed in claims 13-18 are useful as thickeners, viscosifiers, stabilisers for emulsions and starting materials for further functionalisation.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 00/00117

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12P1/00	C12P19/00	C12P13/00	C12P7/24	C07C45/29
	C07C45/32	C07C45/38	C07C45/39	C07H1/00	C08B1/00
	C07H3/00	C07H5/04	C08L1/00	C08L3/00	

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C07C C07H C08B C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CHEMICAL ABSTRACTS, vol. 100, no. 25, 18 June 1984 (1984-06-18) Columbus, Ohio, US; abstract no. 208639, SEMME LHACK, M. F. ET AL: "Oxidation of alcohols to aldehydes with oxygen and cupric ion, mediated b nitrosonium ion" XP002139628 abstract &amp; J. AM. CHEM. SOC. (1984), 106(11), 3374-6 ,</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 August 2000

Date of mailing of the international search report

24.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Douschan, K

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 00/00117

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOBBITT J M ET AL: "ORGANIC NITROSINIUM SALTS AS OXIDANTS IN ORGANIC CHEMISTRY" HETEROCYCLES, XX, XX, vol. 27, no. 2, 1 January 1988 (1988-01-01), pages 509-533, XP000609709 ISSN: 0385-5414 see especially Table 1 the whole document	1-12
X	NOOY DE A E J ET AL: "HIGHLY SELECTIVE TEMPO MEDIATED OXIDATION OF PRIMARY ALCOHOL GROUPS IN POLYSACCHARIDES" RECUEIL DES TRAVAUX CHIMIQUES DES PAYS-BAS, NL, ELSEVIER SCIENCE PUBLISHERS. AMSTERDAM, vol. 113, no. 3, 1 March 1994 (1994-03-01), pages 165-166, XP000560836 ISSN: 0165-0513 the whole document	1-18
X	NOOY DE A E J ET AL: "ON THE USE OF STABLE ORGANIC NITROXYL RADICALS FOR THE OXIDATION OF PRIMARY AND SECONDARY ALCOHOLS" SYNTHESIS, DE, GEORG THIEME VERLAG. STUTTGART, 1 October 1996 (1996-10-01), pages 1153-1174, XP002072173 ISSN: 0039-7881 the whole document	1-12
X, P	WO 99 23117 A (VALTON TEKNILLINEN TUTKIMUSKESKUS) 14 May 1999 (1999-05-14) the whole document	1-12
X	US 3 632 802 A (J. N. BEMILLER ET AL.) 4 January 1972 (1972-01-04) claims 1,8; example 5	13-18
X	US 5 747 658 A (INSTITUT VOOR AGROTECHNOLOGISCH ONDERZOEK) 5 May 1998 (1998-05-05) claims 10,11; example 1	13-18
A	EP 0 124 439 A (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE) 7 November 1984 (1984-11-07) the whole document	13-18



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 00/00117

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9923117	A	14-05-1999	FI 974139 A AU 1035199 A	05-05-1999 24-05-1999
US 3632802	A	04-01-1972	NONE	
US 5747658	A	05-05-1998	NL 9301905 A AU 1123595 A CA 2175794 A EP 0726916 A FI 961905 A JP 9500414 T WO 9512619 A	01-06-1995 23-05-1995 11-05-1995 21-08-1996 03-07-1996 14-01-1997 11-05-1995
EP 0124439	A	07-11-1984	FR 2545101 A DE 3461938 D JP 59205949 A US 4672034 A	02-11-1984 12-02-1987 21-11-1984 09-06-1987

09/1944182  
5060

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
31 August 2000 (31.08.2000)

PCT

(10) International Publication Number  
**WO 00/50621 A3**

(51) International Patent Classification<sup>7</sup>: C12P 1/00, 19/00, 13/00, 7/24, C07C 45/29, 45/32, 45/38, 45/39, C07H 1/00, C08B 1/00, C07H 3/00, 5/04, C08L 1/00, 3/00

(74) Agent: JORRITSMA, Ruurd; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL).

(21) International Application Number: PCT/NL00/00117

(22) International Filing Date: 24 February 2000 (24.02.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
99200536.3 24 February 1999 (24.02.1999) EP

(71) Applicant (for all designated States except US): NEDERLANDSE ORGANISATIE VOOR TOEGEPAST-NATUURWETENSCHAPPELIJK ONDERZOEK TNO [NL/NL]; Schoemakersstraat 97, NL-2628 VK Delft (NL).

(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (for US only): JETTEN, Jan, Matthijs [NL/NL]; Costerlaan 3 B, NL-3701 JL Zeist (NL). VAN DEN DOOL, Ronald, Tako, Marinus [NL/NL]; Dalkruid 1, NL-4102 KR Culemborg (NL). VAN HARTINGSVELDT, Wim [NL/NL]; Victor Hortastraat 27, NL-3822 VM Amersfoort (NL). VAN WANDELEN, Mario, Tarcisius, Ragmandus [NL/NL]; Rooseveltlaan 14, NL-3705 PG Zeist (NL).

Published:

— with international search report

(88) Date of publication of the international search report:  
8 November 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PROCESS FOR PRODUCING NITROSONIUM IONS FROM A NITROXYL COMPOUND, THEIR APPLICATION IN THE SELECTIVE OXIDATION OF PRIMARY ALCOHOLS, AND NOVEL CARBOHYDRATE ALDEHYDES

(57) Abstract: A process for producing aldehydes, and/or carboxylic acids is described, in which a primary alcohol, especially a carbohydrate, is oxidised using a catalytic amount of a nitrosonium compound obtained by oxidising a nitroxyl compound in the presence of an enzyme compound capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per molecule.

WO 00/50621 A3

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/NL 00/00117

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12P1/00	C12P19/00	C12P13/00	C12P7/24	C07C45/29
	C07C45/32	C07C45/38	C07C45/39	C07H1/00	C08B1/00
	C07H3/00	C07H5/04	C08L1/00	C08L3/00	

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C07C C07H C08B C08L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, WPI Data, PAJ

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 100, no. 25, 18 June 1984 (1984-06-18) Columbus, Ohio, US; abstract no. 208639, SEMELHACK, M. F. ET AL: "Oxidation of alcohols to aldehydes with oxygen and cupric ion, mediated b nitrosonium ion" XP002139628 abstract & J. AM. CHEM. SOC. (1984), 106(11), 3374-6 ,  — -/--	1-12

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 August 2000

Date of mailing of the international search report

24.08.00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Douschan, K

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : C12P 1/00, 19/00, 13/00, 7/24, C07C 45/29, 45/32, 45/38, 45/39, C07H 1/00, C08B 1/00, C07H 3/00, 5/04, C08L 1/00, 3/00	<b>A2</b>	(11) International Publication Number: <b>WO 00/50621</b> (43) International Publication Date: 31 August 2000 (31.08.00)
--	-----------	---

(21) International Application Number: PCT/NL00/00117

(22) International Filing Date: 24 February 2000 (24.02.00)

(30) Priority Data:  
99200536.3 24 February 1999 (24.02.99) EP(71) Applicant (for all designated States except US):  
NEDERLANDSE ORGANISATIE VOOR  
TOEGEPAST-NATUURWETENSCHAPPELIJK ON-  
DERZOEK TNO [NL/NL]; Schoemakersstraat 97,  
NL-2628 VK Delft (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): JETTEN, Jan, Matthijs  
[NL/NL]; Costerlaan 3 B, NL-3701 JL Zeist (NL). VAN  
DEN DOOL, Ronald, Tako, Marinus [NL/NL]; Dalkruid 1,  
NL-4102 KR Culemborg (NL). VAN HARTINGSVELDT,  
Wim [NL/NL]; Victor Hortastraat 27, NL-3822 VM Amers-  
foort (NL). VAN WANDELEN, Mario, Tarcisius, Ragman-  
dus [NL/NL]; Roosenveltlaan 14, NL-3705 PG Zeist (NL).(74) Agent: JORRITSMA, Ruurd; Nederlandsch Octrooibureau,  
Scheveningsweg 82, P.O. Box 29720, NL-2502 LS The  
Hague (NL).(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG,  
BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE,  
ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP,  
KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,  
MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU,  
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,  
US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE,  
LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM,  
AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT,  
BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GW, ML, MR, NE, SN, TD, TG).**Published***Without international search report and to be republished  
upon receipt of that report.*

(54) Title: PROCESS FOR SELECTIVE OXIDATION OF PRIMARY ALCOHOLS AND NOVEL CARBOHYDRATE ALDEHYDES

## (57) Abstract

A process for producing aldehydes, and/or carboxylic acids is described, in which a primary alcohol, especially a carbohydrate, is oxidised using a catalytic amount of a nitrosonium compound obtained by oxidising a nitroxyl compound in the presence of an enzyme compound capable of oxidation. Further described are oxidised carbohydrates containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per molecule.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**Process for selective oxidation of primary alcohols and novel carbohydrate aldehydes**

[0001] The invention relates to the production of nitrosonium ions (oxoammonium ions) by oxidation of nitroxyl radicals, especially 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO). The nitrosonium ions can be used as a catalytic oxidising agent for the selective oxidation of primary alcohols to aldehydes.

[0002] Such a process in which TEMPO is reoxidised by chemical means is known from a review by De Nooy in *Synthesis* 1996, 1153-1174 and from WO 95/07303.

[0003] It was found according to the invention that oxidation of alcohol functions, especially primary alcohol functions, can be carried out without using chlorine-based oxidising agents and with the use of hydrogen peroxide or oxygen as the ultimate oxidising agent. The oxidation according to the invention is performed using enzymes and/or metal complexes. This oxidation, when carried out on primary alcohols, surprisingly results in formation of aldehydes, if desired without substantial further oxidation to carboxylic groups using appropriate conditions. The aldehydes may be present in the (hemi)acetal form and related structures. An adaptation of the oxidation process of the invention can be used to oxidise secondary alcohols, especially carbohydrates, to keto derivatives. The process of the invention is further defined by the characterising features of the appending claims.

[0004] The non-prepublished International patent applications WO 99/23117 and WO 99/23240 describe the oxidation of cellulose or starch, respectively, using an oxidative enzyme such as laccase with oxygen and TEMPO mediation. The laccase/TEMPO oxidation of cellulose resulted in the presence of a low and unspecified level carboxyl and carbonyl groups, while the laccase/TEMPO oxidation of starch was reported to yield a product having 1 carboxyl group and 3 aldehyde groups per 100 glucose units; no method of determining aldehyde content was given.

[0005] In the following description, reference is made to TEMPO only for the sake of simplicity, but it should be understood that other suitable nitroxyls, i.e. organic nitroxyl compounds lacking  $\alpha$ -hydrogen atoms, such as 2,2,5,5-tetramethylpyrrolidine-N-oxyl (PROXYL), 4-hydroxy-TEMPO, 4-acetamido-TEMPO and derivatives thereof and those described in WO 95/07303 can be substituted for TEMPO. These di-tert-alkyl nitroxyls are especially suitable for selectively oxidising primary alcohols to aldehyde functions, in particular in the presence of secondary alcohol functions that should not be oxidised. Less sterically hindered nitroxyls, such as 4,4-dimethyloxazolidine-N-oxyl (DOXYL), are suitable for preferentially oxidising secondary alcohols to keto functions, for example in the production of keto cellulose or keto starch. The active oxidising species is the



nitrosonium ion (oxoammonium ion  $>\text{N}^+=\text{O}$ ), that is produced in situ by oxidation of the corresponding hydroxylamine and nitroxyl radical. If desired, the reaction can be performed in two steps, the production of the nitrosonium ion being the first and the oxidation of the alcohol function being the second.

5 [0006] A catalytic amount of nitroxyl is preferably 0.1-25% by weight, based on the primary alcohol, or 0.1-25 mol% with respect to the primary alcohol. The nitroxyl may also be immobilised, e.g. by coupling of the hydroxyl group of 4-hydroxy-TEMPO to a suitable carrier, or in the form of a polymeric nitroxyl such as:

10  $-\text{[(CH}_3)_2\text{C-NO-C(CH}_3)_2\text{-A-]}_n-$ , wherein A may be an alkylene group and/or a heteroatom, and n is a number from e.g. 10 up to several hundreds.

[0007] The process of the invention can be used for the oxidation of primary alcohols initially to the corresponding aldehydes. If required the primary products can be further oxidised to the corresponding carboxylic acids by using known oxidising agents such as hypochlorite, chlorite, hydrogen peroxide or by using TEMPO-mediated oxidation under  
15 more vigorous conditions such as an increased temperature e.g. from 40-80 °C, or for prolonged exposure to the reaction conditions. Alternatively, the aldehyde/carboxylic acid ratio can be increased by using relative low pH's (e.g. pH 3-7), by controlled addition of oxidising agent, by lowering the oxygen concentration, or by first preparing the nitrosonium ion solution (two-step process).

20 [0008] The present process is especially favourable for the selective oxidation of primary hydroxyl groups in alcohols having a secondary alcohol function in addition to the primary alcohol, such as 1,6-octanediol, 1,9-octadecanediol, steroid hormones, sugar alcohols, glycosides (flavour precursors), and in particular carbohydrates having primary alcohol functions. The carbohydrates may be monosaccharides, such as glucose, fructose,  
25 disaccharides, such as sucrose, maltose, lactose, oligosaccharides and polysaccharides. The oligo- and polysaccharides may be of any type, e.g. glucans such as starch, starch components (i.e. amylose, amylopectine, dextrins), pullulan ( $\alpha$ -1,4- $\alpha$ -1,4- $\alpha$ -1,6-glucan), cellulose (in particular non-wood), chitin, lichenin etc., furanofructans such as inulin and levan, galactans, arabinogalactans, furanoid pentosans (xylans), (galacto)mannans (guar,  
30 locust bean gum), bacterial exopolysaccharides (EPS) and the like and derivatives of such carbohydrates, such as hydrolysates. These oligo- and polysaccharides include hetero-saccharides, i.e. those which have different structural units, even if those different units themselves may not have primary hydroxyl groups such as uronic acid units, e.g. in xanthan and carbohydrates derived from algae. The carbohydrates to be oxidised according  
35 to the invention include glycosides and other protected carbohydrates. Further examples are glyconic acids, such as lactobionic acid delta-lactone, that can be oxidised to glycaric acids

and the like.

[0009] A distinct group of compounds suitable for oxidation with the present process consists of hydroxyalkylated carbohydrates such as hydroxypropyl cellulose, hydroxyethyl starch or hydroxyethylinulin, which result in an alternative way for producing formylalkyl carbohydrates. Other suitable carbohydrate substrates in which at least a part of the (6-) hydroxymethyl groups are intact, include for example (2- and 3-) carboxymethyl carbohydrates.

[0010] The oxidation of carbohydrates containing primary hydroxyl groups results in the corresponding carbohydrates containing aldehydes and, if desired, to carboxylic acids, with intact ring systems. Examples include  $\alpha$ -1,4-glucan-6-aldehydes,  $\beta$ -1,4-glucan-6-aldehydes,  $\beta$ -2,1-fructan-6-aldehydes and  $\beta$ -2,6-fructan-1-aldehydes. These products are useful intermediates for functional carbohydrates wherein the aldehyde groups are further reacted with e.g. amine compounds and the like. They are also useful intermediates for crosslinked carbohydrates, in which the aldehyde groups are further reacted with e.g. diamine reagents.

[0011] The catalysts to be used according to the invention are oxidoreductases or other enzymes that are capable of oxidation in the presence of a suitable redox system. Oxidoreductases, i.e. enzymes capable of oxidation without the presence of further redox systems, to be used in the process of the invention include peroxidases and oxidases, in particular polyphenol oxidases and laccase. Certain hydrolases, such as phytase and lipases, can be used when a further redox system is present such as a metal complex, e.g. vanadate. For example, lipases are found to be effective catalysts for selective oxidation of primary alcohol functions with TEMPO / hydrogen peroxide / copper in the presence of an organic, in particular a C<sub>1</sub>-C<sub>6</sub> carboxylic acid (e.g. acetic acid). Instead of complete enzymes, so-called "synzymes", i.e. transition metal complexes mimicking enzymes can be used. Such complexes comprise e.g. vanadium, manganese, iron, cobalt, nickel or copper with complexing agents, in particular polyamines, such as 2,2'-bipyridyl, phenanthroline, tetramethylethylenediamine, pentamethyldiethylenetriamine and their cyclic counterparts such as 1,4,7-trimethyl-1,4,7-triazonane, and histidine and its oligomers. The metal-assisted enzymes require hydrogen peroxide, alkyl and ar(alk)yl hydroperoxides (such as tert-butyl hydroperoxide) or chlorite as an ultimate electron acceptor.

[0012] Peroxidases (EC 1.11.1.1 - 1.11.1.11) that can be used according to the invention include the peroxidases which are cofactor-independent, in particular the classical peroxidases (EC 1.11.1.7). Peroxidases can be derived from any source, including plants, bacteria, filamentous and other fungi and yeasts. Examples are horseradish peroxidase, soy-hull peroxidase, myeloperoxidase, lactoperoxidase, *Arthromyces* and *Coprinus* peroxidases. Several peroxidases are commercially available. The peroxidases require



hydrogen peroxide as an electron acceptor.

[0013] Polyphenol oxidases (EC 1.10.3.1) include tyrosinases and catechol oxidases, such as lignine peroxidase. Suitable polyphenol oxidases may be obtained from fungi, plants or animals. The polyphenol oxidases require oxygen as an electron acceptor.

5 Laccases (EC 1.10.3.2) are sometimes grouped under the polyphenol oxidases, but they can also be classified as a distinct group, sometimes referred to as p-diphenol oxidases. Laccases can be derived from plant sources or from microbial, especially fungal, sources, e.g. of the species *Trametes versicolor*. The use of recombinant laccases can be advantageous. The laccases also require oxygen as an electron acceptor.

10 [0014] The process of the invention can be performed under relatively mild conditions, e.g. at a pH between 2 and 10, and at a temperature between 15 and 60°C (both depending on the particular enzyme or metal complex). The reaction medium can be an aqueous medium, or a homogeneous mixed medium, e.g. of an alcohol/water or an ether/water mixture, or a heterogeneous medium, e.g. a mixture of water and a water-immiscible  
15 organic solvent such as a hydrophobic ether, a hydrocarbon or a halogenated hydrocarbon. In the latter case, the enzyme and/or the nitroxyl and the oxidising agent may be present in the aqueous phase and the alcohol substrate and the aldehyde or ketone product may be present in the organic phase. If necessary, a phase transfer catalyst may be used. This type of reaction is suitable e.g. for the oxidation of steroids, such as the selective oxidation of  
20 19-hydroxy steroids, and the introduction of aldehyde and/or carboxylic groups into other sensitive compounds such as flavour compounds. The reaction medium can also be a solid/liquid mixture, in particular when the enzyme or the nitroxyl are immobilised on a solid carrier. A heterogeneous reaction medium may be advantageous when the substrate or the product is relatively sensitive or when separation of the product from the other  
25 reagents may present difficulties.

[0015] The invention also pertains to novel carbohydrate oxidation products and derivatives thereof obtainable with the process of the invention. These include polysaccharides in which at least 1 hydroxymethyl per 100, especially per 50 or even per 25, monosaccharide units has been converted to a carbaldehyde group, whether or not in  
30 hemiacetal or similar form, with the proviso that on average each molecule contains at least 1 carbaldehyde group other than a possible (hemiacetalised) aldehyde group at the reducing end of an oligo- or polysaccharide. When the carbohydrate is starch, the degree of oxidation is at least one carbaldehyde group per 25 anhydroglucose units. The carbaldehyde group is preferably present in chain (backbone) units, rather than in branch  
35 units. Not included in this at least carbaldehyde group per 100 (50, 25) units are carbaldehyde groups derived from terminal galactose units, which are obtainable by

oxidation with galactose oxidase. The novel products include glycoside derivatives, i.e. products which, in addition to an acetalised end group have at least one carbaldehyde group obtainable by oxidation of non-galactose hydroxymethylene groups.

[0016] In the products of the invention, the monosaccharide rings that carry the carbaldehyde group are largely intact, and the number of aldehyde groups is greater, especially more than two times greater, than the number of carboxyl groups (other than introduced carboxyalkyl groups). Such products are not easily produced by prior art oxidation methods, which invariably lead to at least partial further oxidation to carboxyl groups. The only common carbohydrate derivatives having a predominant content of aldehyde groups are periodate-type oxidation products of starch, cellulose and the like, in which the rings bearing the aldehyde groups are broken. The aldehyde carbohydrates covered by the present invention are in particular of the non-cellulose type. The products obtainable according to the invention may contain, in addition to the aldehyde groups, other functional groups, especially carboxyl groups obtained by further oxidation or by carboxyalkylation (e.g. reaction with chloroacetic acid).

[0017] The novel derivatives of the invention are very suitable as thickeners, viscosifiers, stabilisers for emulsions and the like, and especially as starting materials for further functionalisation, especially with alcohols, amines, and other agents capable of coupling with an aldehyde function. Such agents include crosslinking agents (diamines, diols and the like), which can be used to crosslink the carbohydrates or to couple them to amino acids, proteins, active groups etc.

[0018] The process of the invention can also advantageously be used for modifying biopolymers such as starch or cotton cellulose, to allow derivatisation (e.g. dyeing of textile, strengthening of textile fibres and anti-pilling) or to adapt viscosity and other physical or chemical properties, for example to modify dietary fibres including fructans, mannans, cellulose etc.

[0019] The invention also pertains to derivatives obtained by coupling of the aldehyde carbohydrates described above with e.g. amines, especially by reductive amination, to produce imino or amino derivatives of carbohydrates as defined in the appending claims. Also, the aldehyde carbohydrates can be reacted acetalised with hydroxy-functionalised compounds, e.g. glycolic acid, for further derivatisation.

#### **Examples: General**

[0020] Uronic acid (6-COOH of hexopyranose units) contents were determined using the Blumenkrantz et al. method (*Anal. Biochem.* (1973) 54, 484), using boric acid (0.0125 M) in concentrated sulphuric acid, adding 3-hydroxybiphenyl and measuring the

extinction is measured at 520 nm.

[0021] Aldehyde contents were determined either by a subtractive method (determining the uronic acid content before and after of oxidation of aldehydes with chlorite and hydrogen peroxide), or by addition of hydroxylamine hydrochloride to produce an oxime and back-titration of liberated hydrochloric acid, or by  $^{13}\text{C}$  NMR spectroscopy (intensity of C6 signal of aldehyde with respect to C1 of anhydroglucose unit, or intensity of C6 (C=N) in the oxime).

**Example 1: Production of 6-aldehyde starch using horse radish peroxidase**

10 [0022] Two grams of starch were gelatinised in 100 ml of water at 100°C. The solution obtained was cooled to 22°C. To this solution were added 25 mg TEMPO (0.13 mmol) and 40 mg of peroxidase (HRPO). The pH was adjusted to 5 with acetic acid (0.1 M). A hydrogen peroxide solution (1.5 ml 30% in 50 ml) was added drop-wise (2 ml per h). No pH adjustment was necessary. After 25 h a sample was analysed by addition of  
15 hydroxylammonium chloride. According to this indirect analysis, 30% of C6-aldehyde starch was formed, which was confirmed by  $^{13}\text{C}$  NMR.

**Example 2: Oxidation of pullulan with laccase**

[0023] Through a solution of 1.84 g of pullulan (11.5 mmol anhydroglucose units) 17  
20 mg of *Trametes versicolor* laccase VIIIb (expressed in recombinant *E. coli*, Wacker Chemie) and 25 mg of TEMPO in 100 ml water, oxygen gas was bubbled. The pH of the solution (6.1) decreased gradually to 4.5 after 24 hours. The aldehyde content of the solution determined by reaction with hydroxylamine hydrochloride was 1.1 mmol. The uronic acid content was 24%. To oxidise the aldehyde groups to carboxylic acid groups,  
25 the solution was treated with sodium chlorite and hydrogen peroxide. After treatment the uronic acid was increased to 32%. Based on the oxidisable groups the yields are 36 and 48 %, respectively. The solution was poured out into ethanol. A white precipitate was formed, which after one day was collected by filtration and dried in vacuum. The uronic acid content of this material was 25%.

30

**Example 3: Oxidation of pullulan with laccase**

[0024] A solution of 1.84 g pullulan (11.5 mmol), 100 mg 4-acetamido-TEMPO, and 18 mg laccase (*T. versicolor*) was prepared. The mixture was buffered with sodium acetate / acetic acid buffer (0.05M). The initial pH of the solution was 6.1. This mixture  
35 was exposed to oxygen gas in a closed system. After one day reaction 24 ml of oxygen was consumed. To bring the pH to its original value 2 ml 0.5 M NaOH was added. The

reaction was continued for another day, resulting in the consumption of 20 ml of oxygen gas. The final pH was 5.1. The pH was adjusted again by addition of 1.2 ml NaOH (0.5 M). 15 mg laccase was added and the reaction was allowed to proceed for two days. After this period the pH was 4.5 and 30 ml oxygen gas was consumed. To bring the pH to 6, 3 ml 0.5 NaOH had to be added. To the solution 0.2 ml hydrogen peroxide (30% w/w) and 250 mg sodium chlorite were added. After one day reaction the uronic acid content was measured. The yield of uronic acid before oxidation with sodium chlorite was 550 mg (26%) and after 695 mg (33%).

10 **Example 4: Preparation of the nitrosonium salt of TEMPO using laccase**

[0025] A solution of TEMPO nitrosonium ion was made with laccase as follow. 6.9 g TEMPO was dissolved in 1 l demi water. 200 mg laccase VIIIb from *T. versicolor* (Wacker) was suspended in 20 ml demi water. After stirring the enzyme solution for 10 minutes, the supernatant after centrifugation (5 min 1500xg) was desalted using a P6 column. The desalted material was added to the TEMPO solution. In approximately 150 minutes under pH stat conditions at pH 5, ambient temperature, aerated with air sparge, 91 % of the TEMPO was converted to nitrosonium, as determined by the consumption of 100.8 ml of HCl (0,4 N) and a shift from a yellow to a more orange colour (the ratio E480/ E430 increases from approximately 0.3 to 1).

20

**Example 5: Oxidation of starch using nitrosonium salt and a UF membrane system.**

[0026] The nitrosonium solution obtained according to example 4 was buffered with 0.2 M acetate at pH 4.5. 2 g native potato starch was gelatinised in 100 ml water and mixed with 100 ml of the buffered nitrosonium solution. The mixture was poured into a 200 ml stirred UF vessel (cut-off 5 kD). Approximately 800 ml of the nitrosonium solution was pumped into the vessel at a rate of 0.5 ml/min. at room temp 20 °C. The permeate indicated a conversion of 50% of the nitrosonium ion back to TEMPO (based on the E480/E430 ratio). After this treatment, the uronic acid content of the starch was found to be 38%.

30

**Example 6: Conversion of starch using oxygen / laccase / TEMPO cycle**

[0027] Starch solutions were prepared by gelatinising Lintner potato starch (Sigma S-2630) in water. The pH was adjusted by addition of 0.2 M succinic acid / succinate buffer. Tempo or 4-acetamido tempo (4acmT) was added. (TEMPO forms a precipitate with starch in some conditions, which dissolves during the process.) Laccase VIIIb from *Trametes versicolor* expressed in *E.coli* (from Wacker Chemie) was suspended at 10

mg/ml 0,2 M succinate buffer pH 6. After centrifugation (5 min 1500g ) for 10 mg laccase 1 ml of the supernatant was added. The oxygen transfer to the solution was enhanced in stirred pressurised vessels A en B. Both vessels contained approximately 100 ml. The area of contact with the gas phase was 70 cm<sup>2</sup> for vessel A and 32 cm<sup>2</sup> for vessel B. The experimental conditions and the results with regard to C<sub>6</sub>-oxidation (aldehyde or carboxylic acid) are summarised in tables 1 and 2. Important parameters for the reaction conditions are:

- oxygen transfer to the solution, pH, temperature, concentration of TEMPO, enzyme and starch .

10 The formation of uronic acids was monitored according to Blumenkrantz. The formation of aldehydes was monitored after oxidation to uronic acids under the following conditions:

To 5 ml sample (20 g/l starch) 0.095 ml 3% H<sub>2</sub>O<sub>2</sub> and 0.5 ml 20 mg/ml sodium chlorite was added. The uronic acid content was measured after 16 h at room temperature.

15

table 1-1. Summary of influences studied in vessel A

air	O <sub>2</sub>	Starch	Laccase	pH	Temp	4acmT <sup>1</sup>	T	Time	% COOH	% ald or hemi <sup>2</sup>
bar	bar	g/l	mg/100ml		°C	g/l	°C	h		
		lintner			o	l				
2		10	100	6	1		25	45	73,4	nd
1,5		10	100	5		5	25	45	54,2	nd
	4	10	100	6	4		30	15	94,9	nd
2		10	100	6	4		30	15	100,0	nd
	1	10	100*	5,3		6	30	15	78,8	nd
2		10	10	6	4		30	20	60,3	4,9
	1	10	10*	6	4		30	20	50,0	5,1
	4	10	10	5,3		4	30	20	30,5	11,9
	4	20	10*	4,5		4	30	20	19,7	12,0
2		20	10	4		4	30	20	11,5	12,5

\* the enzyme was pumped into the vessel during 20 h

20 <sup>1</sup> 4acmT = 4-acetamido-TEMPO

<sup>2</sup> aldehyde or hemiacetal thereof

table 1-2 Summary of influences studied in vessel B

O2	Starch	laccase	pH	Temp	4acmT	T	time	% COOH	% ald or
bar	lintner	mg/100ml		o	g/l	°C	h		hemi
	g/l			g/l					
4	10	100	6	4		30	15	94,9	nd
4	10	10	5,3		4	30	20	30,5	11,9
6	20	1	6		4	40	20	7,1	4,2

**Example 7: Oxidation of pullulan by TEMPO / Mn / H<sub>2</sub>O<sub>2</sub>**

In 25 ml of water 250 mg pullulan and 20 mg of TEMPO were dissolved. To this  
 5 solution 25 mg manganese nitrate was added, followed by 100 µl of hydrogen peroxide  
 (3% solution, w/w) and bipyridine solution (5 ml 0.05 M). The reaction was conducted  
 at pH 6.5. At the first day 60 mg (1.8 mmol) hydrogen peroxide was added and after one  
 day 25 mg of uronic acid was formed. During the second day 30 mg hydrogen peroxide  
 was added and the amount of uronic acid was increased to 50 mg. The aldehyde groups  
 10 were converted into carboxylic acid groups with hydrogen peroxide/sodium chlorite the  
 content raised to 90 mg. (D.O. 60%).



## Claims

1. A process for producing nitrosonium ions by oxidising a nitroxyl compound with an oxidising agent, *characterised* in that the nitroxyl compound is oxidised in the presence of an enzyme capable of oxidation and/or in the presence of a metal complex.
2. A process according to Claim 1, wherein the nitroxyl compound is a di-tert-nitroxyl compound, especially 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO).
3. A process according to Claim 1 or 2, wherein the enzyme capable of oxidation is an oxidoreductase.
4. A process according to Claim 3, wherein the enzyme is a peroxidase, especially horse radish, soy-bean, lignin peroxidase or myelo- or lacto-peroxidase, and the oxidising agent is hydrogen peroxide.
5. A process according to Claim 3, wherein the enzyme is a polyphenol oxidase or a laccase and the oxidising agent is oxygen.
6. A process according to Claim 1 or 2, wherein the enzyme is a hydrolase, especially phytase or lipase, in the presence of a metal compound.
7. A process for oxidising a primary alcohol with a nitrosonium ion as a catalyst, *characterised* in that the nitrosonium ion is produced by the process according to any one of Claims 1-6.
8. A process according to Claim 7, wherein the primary alcohol is comprised in a carbohydrate, especially an  $\alpha$ -glucan or fructan or a derivative thereof.
9. A process according to Claim 8, wherein a carbonyl-containing carbohydrate containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per average molecule is produced.
10. A process according to Claim 8 or 9, wherein the carbohydrate is a hydroxy-alkylated carbohydrate or a glycoside or a glyconic acid.
11. A process according to any one of Claims 1-7, wherein the primary alcohol is comprised in a steroid compound.

12. A process for treating textile fibres to introduce aldehyde groups, *characterised* in that the cotton fibres are treated with nitrosonium ion produced by the process according to any one of Claims 1-6.
13. An oxidised carbohydrate, the carbohydrate being selected from disaccharides, oligosaccharides and polysaccharides of the glucan, mannan, galactan, fructan, and chitin types and carbohydrate glycosides, containing at least 1 cyclic monosaccharide chain group carrying a carbaldehyde group per 25 monosaccharide units and per average molecule, or a chemical derivative thereof.
14. An oxidised carbohydrate according to Claim 13, containing at least 5 monosaccharide units per average molecule.
15. An oxidised carbohydrate according to Claim 13 or 14, which contains 1 to 50 cyclic monosaccharide chain group carrying a carbaldehyde group per 50 monosaccharide units and per average molecule.
16. A carbohydrate derivative according to any one of Claims 13-15, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula  $-\text{CH}=\text{N}-\text{R}$  or  $-\text{CH}_2-\text{NHR}$ , wherein R is hydrogen, hydroxyl, amino, or a group  $\text{R}^1$ ,  $\text{OR}^1$  or  $\text{NHR}^1$ , in which  $\text{R}^1$  is  $\text{C}_1$ - $\text{C}_{20}$  alkyl,  $\text{C}_1$ - $\text{C}_{20}$  acyl, a carbohydrate residue, or group coupled with or capable of coupling with a carbohydrate residue.
17. A carbohydrate derivative according to any one of Claims 13-15, in which derivative at least a part of the carbaldehyde groups has been converted to a group with the formula  $-\text{CH}(\text{OR}^3)-\text{O}-\text{CH}_2-\text{COOR}^2$  or  $-\text{CH}(-\text{O}-\text{CH}_2-\text{COOR}^2)_2$ , in which  $\text{R}^2$  is hydrogen, a metal cation or an optionally substituted ammonium group, and  $\text{R}^3$  is hydrogen or a direct bond to the oxygen atom of a dehydrogenated hydroxyl group of the carbohydrate.
18. A carbohydrate according to any one of Claims 13-17, further containing carboxyl and/or carboxymethyl groups.